

**Listing of Claims:**

1-19 (Canceled)

20. (Previously presented) A method of preparing an assay sample for detecting bacteria by a flow cytometer, comprising:

providing a diluent comprising a cationic surfactant, a buffer for maintaining a pH of 2.0-4.5 and an effective amount of a substance capable of reducing nitrite ions and a staining solution comprising a polymethine dye for staining bacteria;

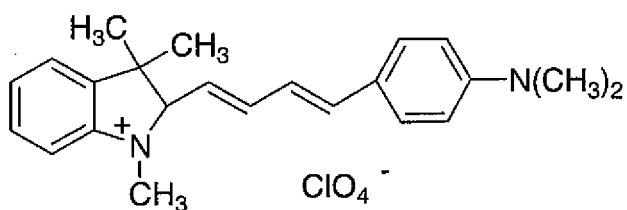
mixing a urine sample with the diluent; and

preparing the assay sample by mixing the mixture of the urine sample and the diluent with the staining solution;

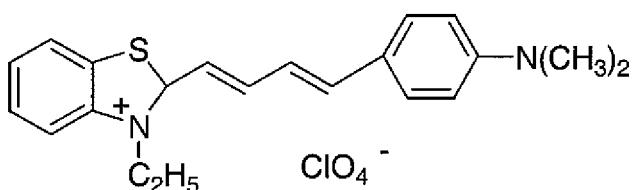
wherein the polymethine dye is at least one selected from the following group consisting of:

(1) Thiazole Orange;

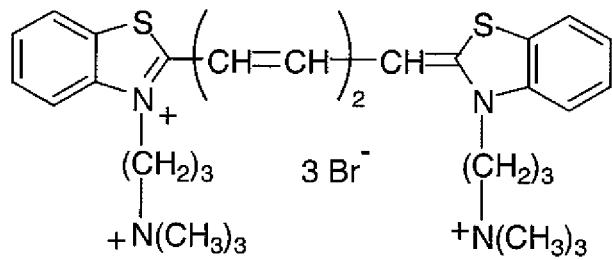
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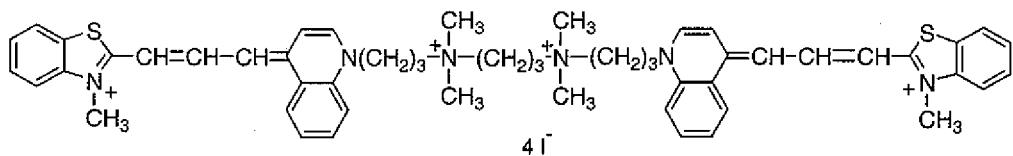
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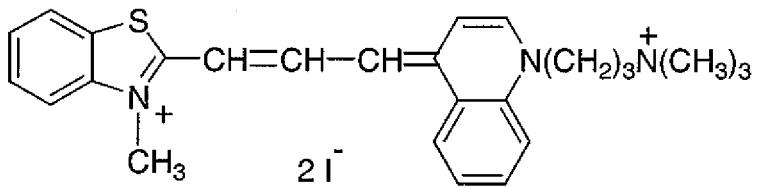
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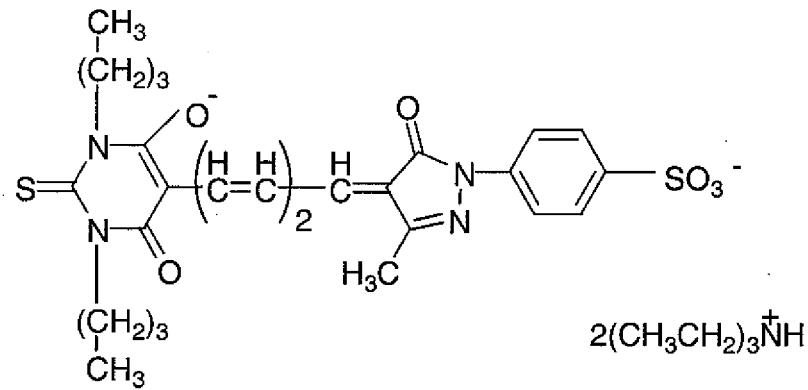
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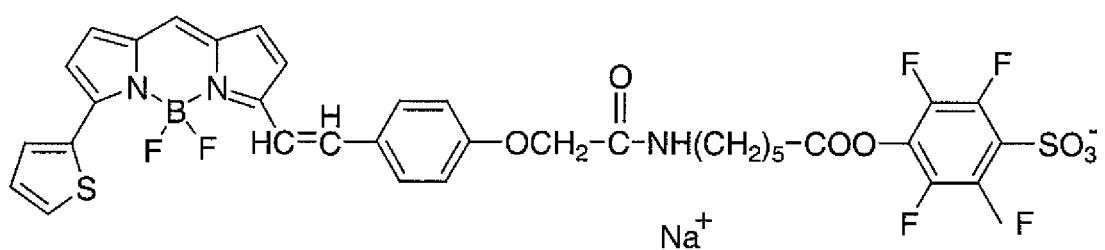
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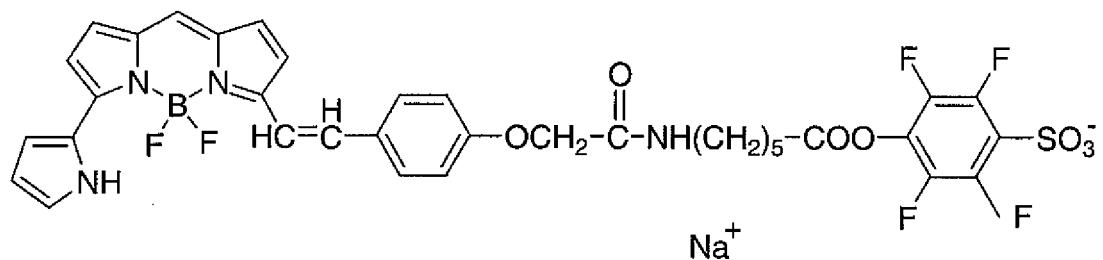
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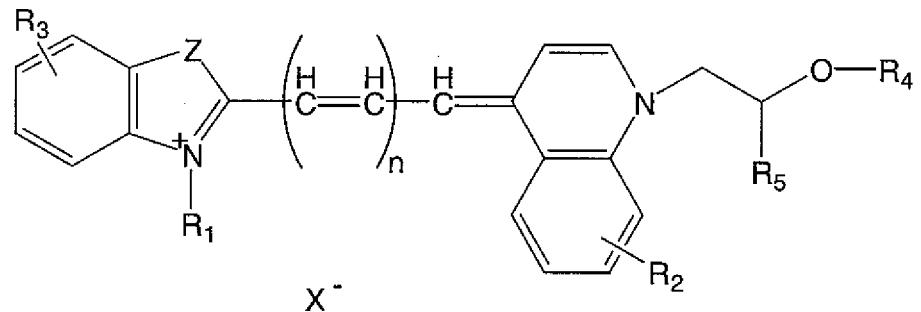
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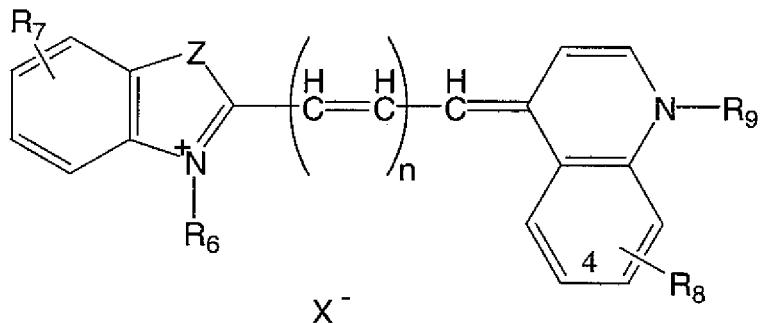


(10) a compound represented by the following general formula:



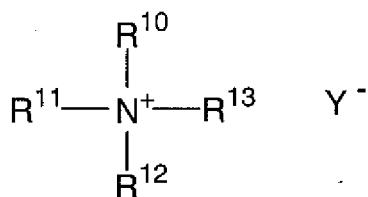
wherein R<sub>1</sub> is a hydrogen atom or a C<sub>1-3</sub> alkyl group; R<sub>2</sub> and R<sub>3</sub> are a hydrogen atom, a C<sub>1-3</sub> alkyl group or a C<sub>1-3</sub> alkoxy group; R<sub>4</sub> is a hydrogen atom, an acyl group or a C<sub>1-3</sub> alkyl group; R<sub>5</sub> is a hydrogen atom or a C<sub>1-3</sub> alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C<sub>1-3</sub> alkyl group; n is 1 or 2; X<sup>-</sup> is an anion; and

(11) a compound represented by the following general formula:



wherein R<sub>6</sub> is a hydrogen atom or a C<sub>1-18</sub> alkyl group; R<sub>7</sub> and R<sub>8</sub> are a hydrogen atom, a C<sub>1-3</sub> alkyl group or a C<sub>1-3</sub> alkoxy group; R<sub>9</sub> is a hydrogen atom, an acyl group or a C<sub>1-18</sub> alkyl group; Z is sulfur, oxygen or a carbon atom having a C<sub>1-3</sub> alkyl group; n is 0, 1 or 2; X<sup>-</sup> is an anion; and

wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R<sup>10</sup> is a C<sub>6-18</sub> alkyl group or a benzyl group; R<sup>11</sup>, R<sup>12</sup> and R<sup>13</sup>, the same or different, are a C<sub>1-3</sub> alkyl group or a benzyl group; Y<sup>-</sup> is a halogen ion.

21. (Previously Presented) The method according to claim 20, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, asparatic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.

22. (Canceled)

23. (Canceled)

24. (Previously Presented) The method according to claim 20, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt,

dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

25. (Canceled)

26. (Previously Presented) The method according to claim 20, wherein the diluent has pH of 2.0-3.0.

27. (Canceled)

28. (Previously Presented) The method according to claim 20, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH,  $\epsilon$ -aminocaproic acid-HCl, fumaric acid-HCl,  $\beta$ -alanine-NaOH and glycine-NaOH.

29. (Previously Presented) The method according to claim 20, wherein the diluent comprises an inorganic salt of either sulfate or nitrate.

30. (Previously Presented) The method according to claim 20, wherein the dye is present at 0.1 to 100 ppm in the assay sample.

31. (Previously Presented) The method according to claim 20, wherein the cationic surfactant exists at 10 to 30000 mg/l in the assay sample.

32. (Canceled)

33. (Canceled)

34. (Canceled)

35. (Previously Presented) The method according to claim 20, wherein the staining solution comprises a water-soluble organic solvent.

36. (Previously Presented) The method according to claim 35, wherein the water-soluble organic solvent is selected from the group consisting of methanol, ethanol and ethylene glycol.

37. (Previously Presented) The method according to claim 35, wherein the water-soluble organic solvent comprises ethylene glycol.

38. (Previously Presented) A method of staining bacteria comprising:  
providing a diluent comprising a cationic surfactant, a buffer for maintaining a pH of 2.0-4.5 and an effective amount of a substance capable of reducing nitrite ions and a staining solution comprising a polymethine dye for staining bacteria;

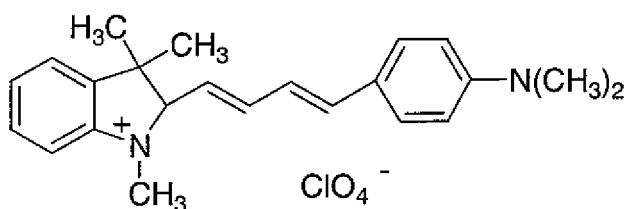
mixing a urine sample with the diluent; and

mixing the mixture of the urine sample and the diluent with the staining solution;

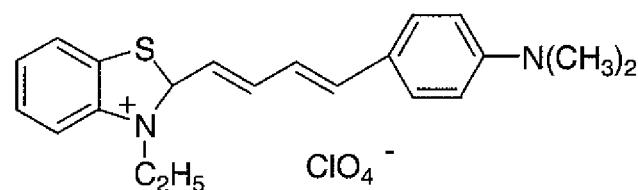
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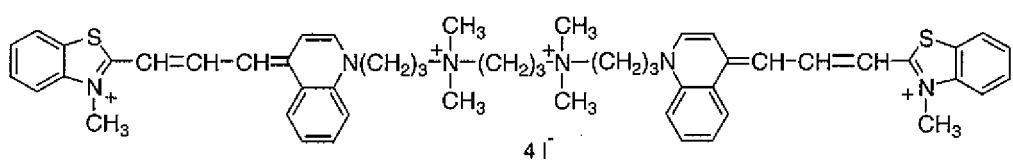
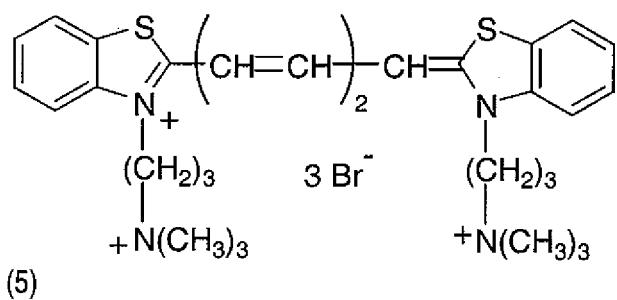
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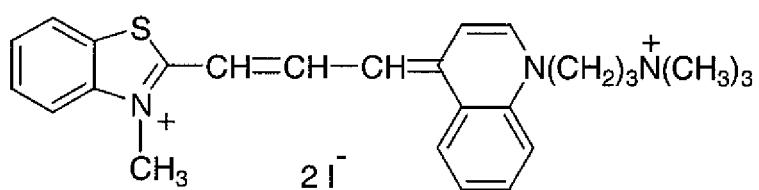
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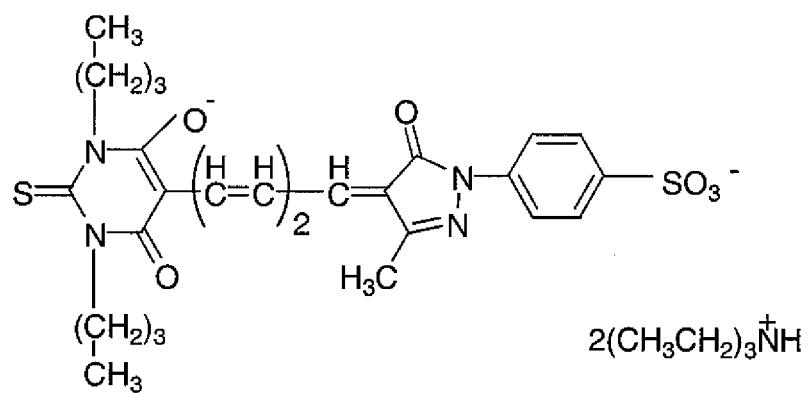
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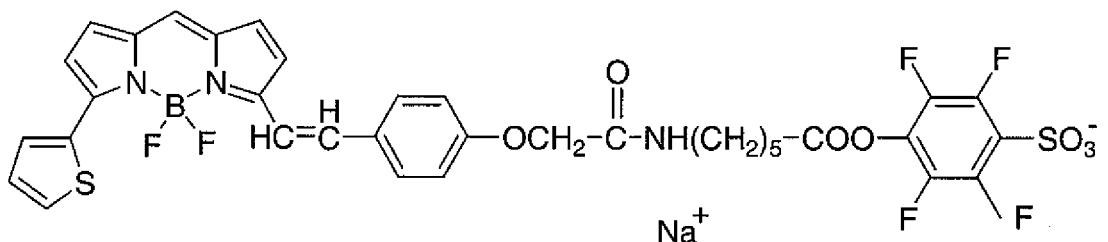
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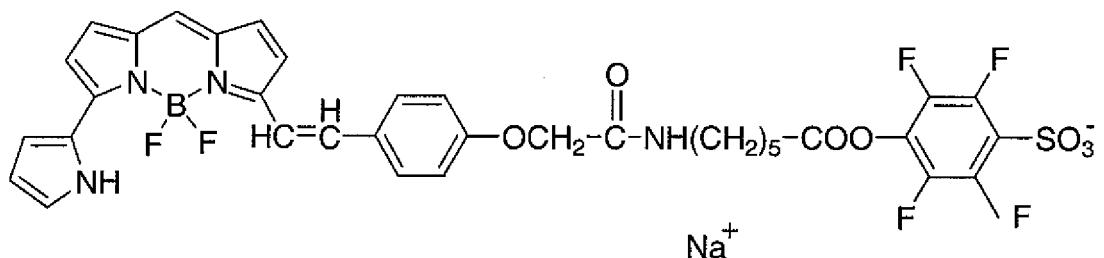
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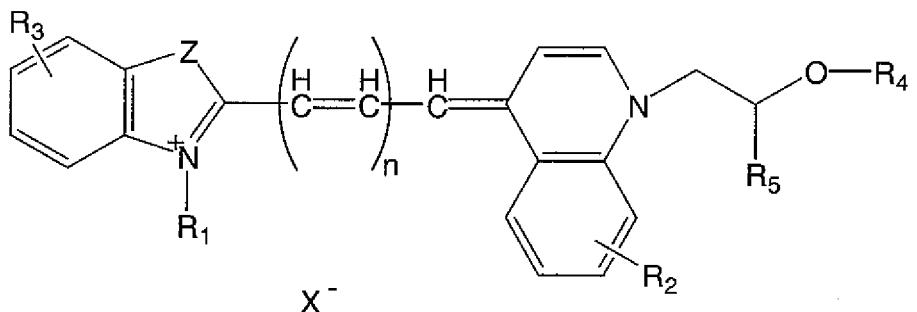
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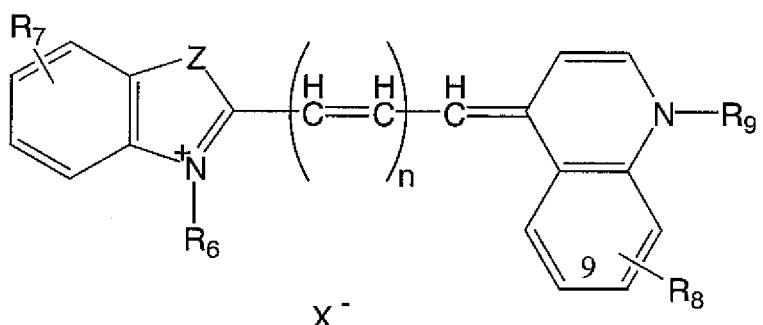


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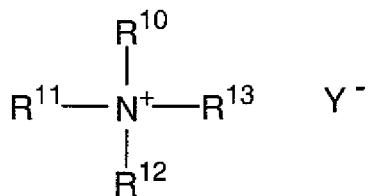
wherein  $\text{R}_1$  is a hydrogen atom or a C<sub>1-3</sub> alkyl group;  $\text{R}_2$  and  $\text{R}_3$  are a hydrogen atom, a C<sub>1-3</sub> alkyl group or a C<sub>1-3</sub> alkoxy group;  $\text{R}_4$  is a hydrogen atom, an acyl group or a C<sub>1-3</sub> alkyl group;  $\text{R}_5$  is a hydrogen atom or a C<sub>1-3</sub> alkyl group which may be substituted;  $Z$  is a sulfur atom, an oxygen atom or a carbon atom substituted with a C<sub>1-3</sub> alkyl group;  $n$  is 1 or 2;  $\text{X}^-$  is an anion; and

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wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R<sup>10</sup> is a C<sub>6-18</sub> alkyl group or a benzyl group; R<sup>11</sup>, R<sup>12</sup> and R<sup>13</sup>, the same or different, are a C<sub>1-3</sub> alkyl group or a benzyl group; Y<sup>-</sup> is a halogen ion.